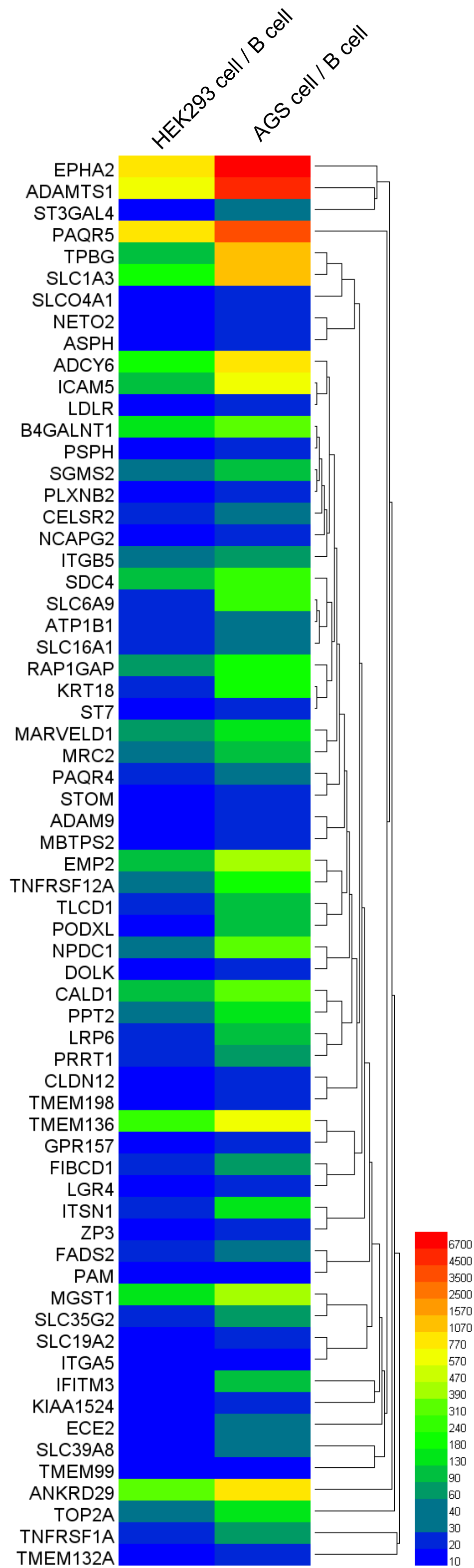
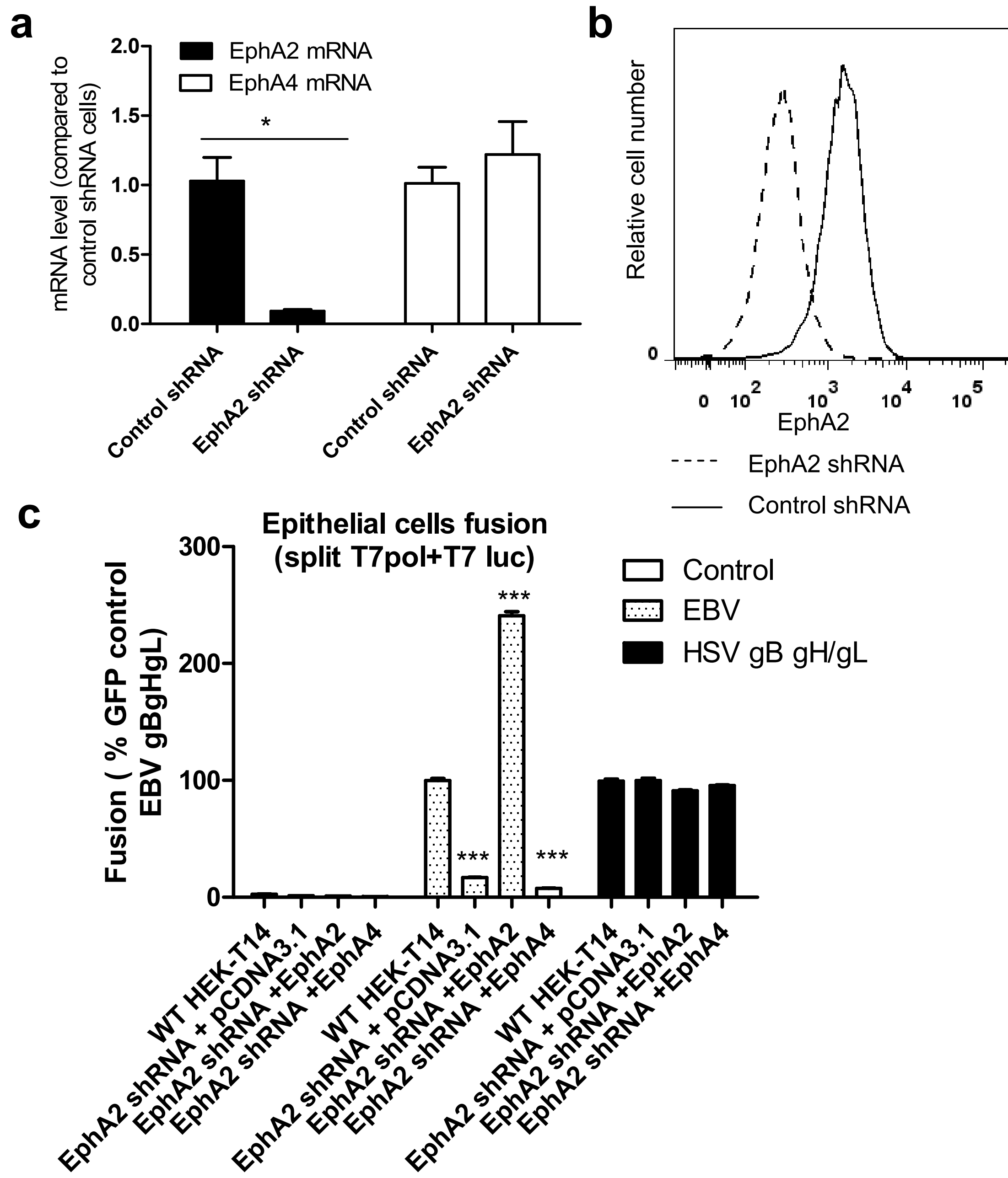
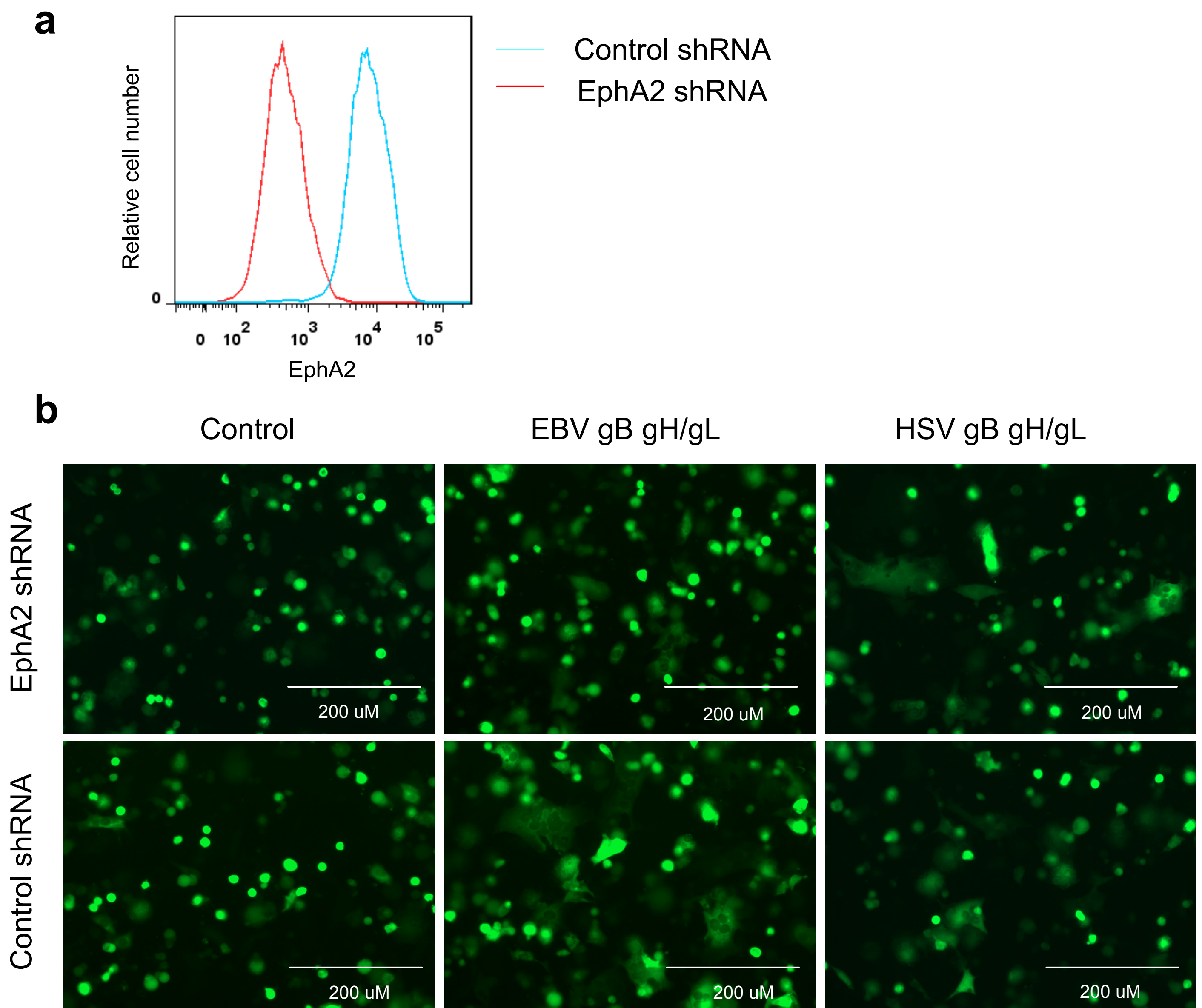
EphA2 is a functional entry receptor for Epstein-Barr Virus.



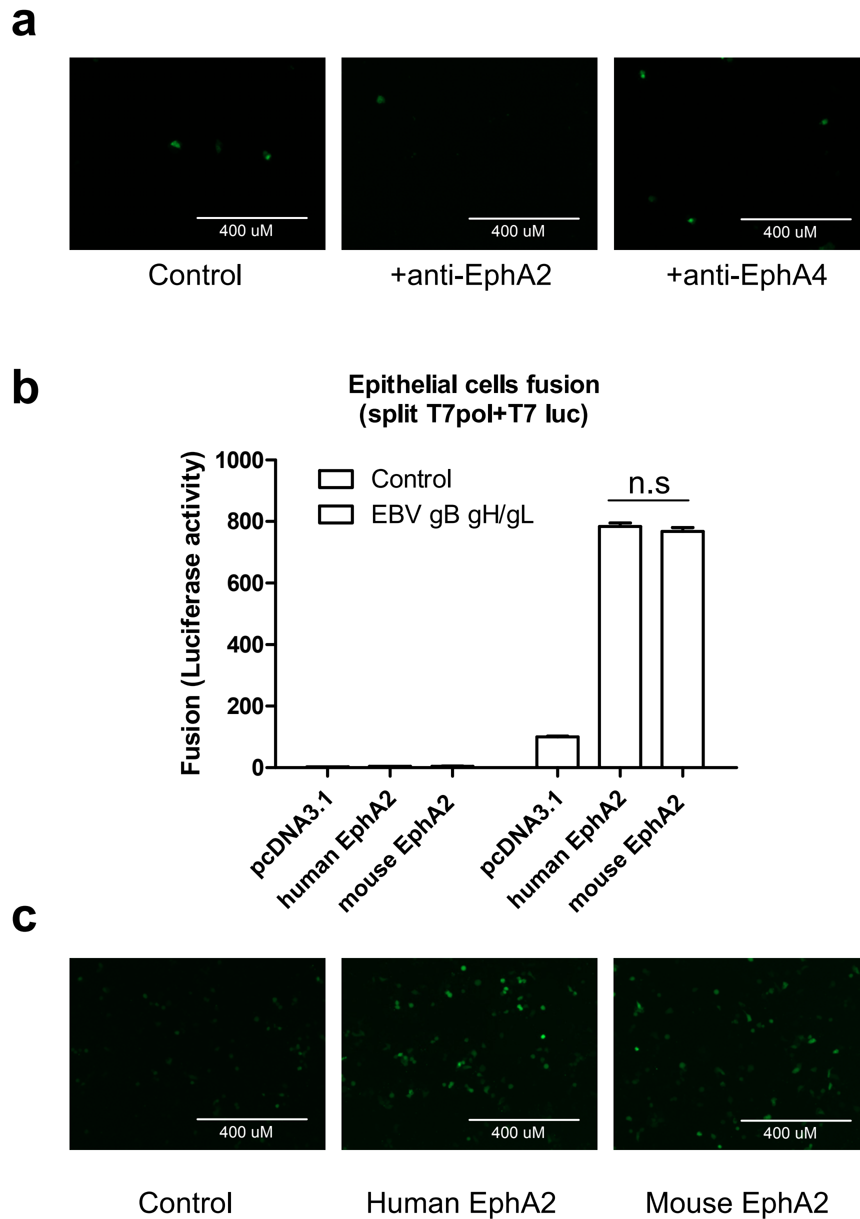
Supplementary Figure 1. Bioinformatics analysis. Panel c in Fig. 1 has been reproduced here using a different analysis.



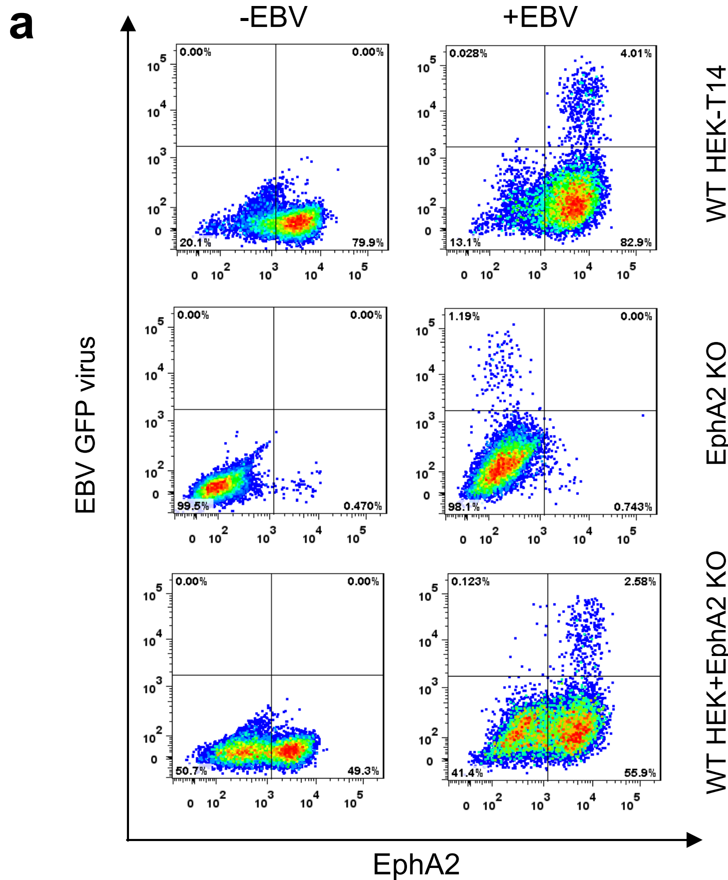
Supplementary Figure 2. EphA2 is essential for EBV fusion of HEK 293 cells. **a** and **b**, Stable silencing of EphA2 expression in HEK293-T14 cells. HEK293-T14 cells were infected with lentivirus containing EphA2-specific shRNA constructs or control construct. Levels of EphA2 and EphA4 mRNA in the transduced HEK293-T14 cells were assessed by qRT-PCR (**a**). The bars represent the relative EphA2 and EphA4 mRNA expression that was normalized to GAPDH and the relative level of EphA2 and EphA4 mRNA in the control HEK293-T14 cells was set to 100. . Data are means plus standard errors of the means for three independent experiments. **b**, Cell surface expression of EphA2 was determined by flow cytometry (representative data from two independent experiments). **c**, Virus-free EBV or HSV fusion with EphA2 WT and EphA2 knock-down HEK293-T14 cells or EphA2 knock down cells that overexpress EphA2 or EphA4. The bars represent the fusion activity and data are means plus standard errors of the means for three independent experiments. *P<0.05 in A (Students *t* test). ***P<0.001 vs WT HEK-T14 in C (ANOVA followed by post-hoc Tukey's multiple comparison test).

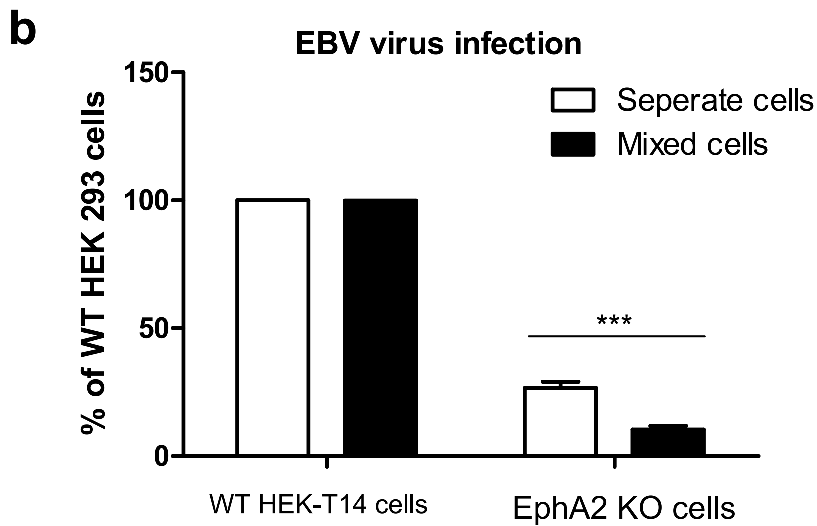


Supplementary Figure 3. EphA2 is essential for EBV fusion of AGS cells. AGS cells were infected with lentivirus containing EphA2-specific shRNA constructs or control construct . (**a**) EphA2 cell surface expression in the transduced AGS cells was assessed by flow cytometry (representative data from two independent experiments). (**b**) Virus-free EBV or HSV fusion with EphA2 WT and EphA2 knock-down cells was determined by syncytia formation assay. Data are representative data from two independent experiments.



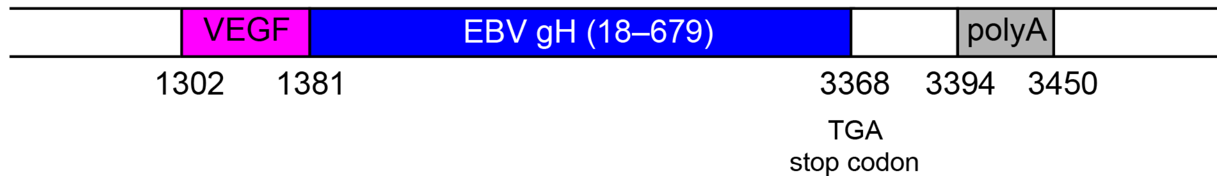
Supplementary Figure 4. EBV infection is blocked by anti EphA2 antibody and murine EphA2 does not function for EBV infection. **a**, 1.5×10^5 Detroit 562 cells cultured on 6.5-mm-diameter Costar Transwell filters, were pre-incubated without or with 2ug/mL anti-EphA2 or anti-EphA4 antibody for one hour and then infected with 400 uL (bottom)+100 mL (top) EBfaV-GFP virus concentrated from 5 mL virus-containing supernatant. 72 hours later, the infected GFP cells were visualized and captured with a EVOS fluorescence microscope
b, Virus-free EBV fusion with HEK 293 cells transfected with pcDNA3.1, human EphA2 or mouse EphA2. The bars represent the fusion activity and data are means plus standard errors of the means for three independent experiments. **c**, HEK 293 cells transfected with pcDNA 3.1, human EphA2 or mouse EphA2 were infected with EBfaV-GFP virus. 72 h post infection, the infected GFP cells were visualized and captured with a EVOS fluorescence microscope. One representative infection data of at least two independent experiments is shown in **a**, and **c**.



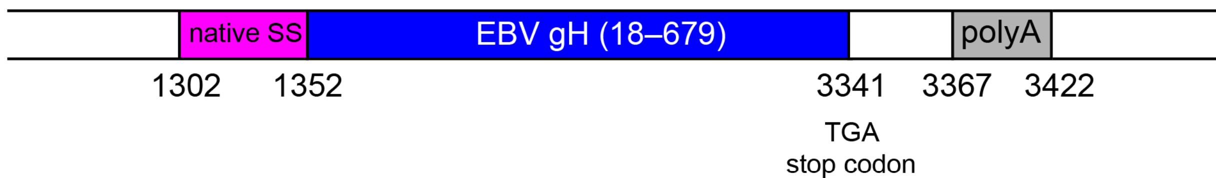


Supplementary Figure 5. EphA2 WT cells compete with EphA2 KO cells for EBV infection. 5x104 EphA2 WT HEK293-T14 cells, 5x10^4 EphA2 KO HEK293-T14 cells or a mixture of 2.5x10^4 EphA2 WT and 2.5x10^4 EphA2 KO HEK293-T14 cells were infected with 100 mL EBfaV-GFP virus concentrated from 1 mL virus-containing supernatant. 72 hours later, the infected GFP cells were analyzed by flow cytometry (**a**). **b**, Quantification of the flow cytomery data from three independent experiments, the bars represent the percentage of infection and infection of WT HEK293 cells was set to 100.*** $P<0.001$ (ANOVA followed by post-hoc Tukey's multiple comparison test).

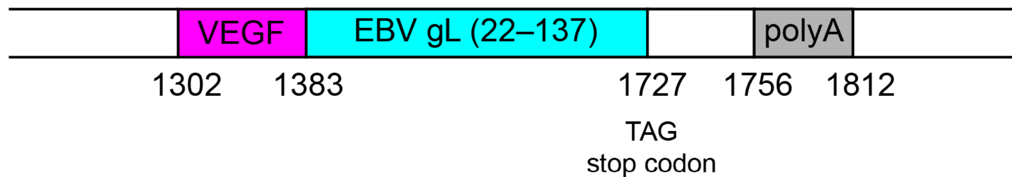
1) EBV gH, pTTVH8G, VEGF SS, 8012 bp



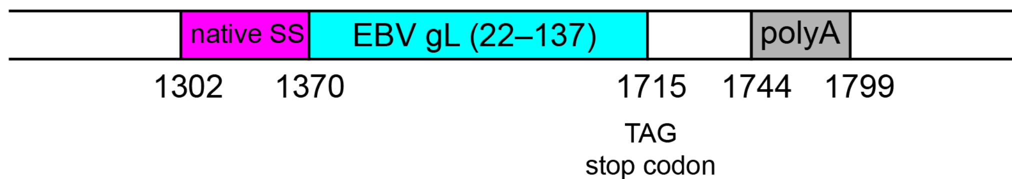
2) EBV gH, pTTVH8G, native SS, 7985 bp



3) EBV gL, pTTVH8G, VEGF SS, 6374 bp



4) EBV gL, pTTVH8G, native SS, 6362 bp



Supplementary Figure 6. Mammalian cell expression plasmids for soluble EBV gH and EBV gL. Empty pTTVH8G vector (National Research Council Canada, NRC license file 11266) was made to express EBV gH and EBV gL by Gibson assembly and used in mammalian cell transient transfections in 1:1 ratio as used here for expressing soluble EBV gHgL protein in HEK 293EBNA1-6E cells (National Research Council Canada, NRC license file 11565). 4 Gibson assembly plasmids as shown here were made and sequence verified (see Methods). Pairs of either VEGF SS or native SS EBV gH and gL plasmids were used for transient transfections and resulted in similar protein yields.

Surface Plasmon Resonance (SPR) kinetic parameters from 1:1 interaction model					
Immobilized Ligand	**Surface density of Ligand**[a]	**Mobile phase (Analyte)**	**On-rate, k_a, $M^{-1}.s^{-1}$ ($X10^3$)**	**Off-rate, k_d, s^{-1}**	**K_D (μM)**
EphA2	4630 RU[b]	EBV gHgL	3.98	0.020	5
EphA4	5650 RU	EBV gHgL	-	-	-

Supplementary Table 5. Surface Plasmon Resonance (SPR) kinetic parameters from 1:1 interaction model

a Ligand density rounded off to the nearest tenth after subtracting RU value of baseline level after deactivation step with ethanolamine for the ligand channel minus the corresponding reference channel (Ch4-Ch1 or Ch3-Ch2).

b RU is Resonance unit or Response unit. One representative data of three independent experiments is shown.